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<p>(21) 国際出願番号 PCT/JP97/02728 (22) 国際出願日 1997年8月6日(06.08.97) (30) 優先権データ 特願平8/208504 1996年8月7日(07.08.96) JP (71) 出願人 (米国を除くすべての指定国について) カルピス株式会社 (CALPIS CO., LTD.)(JP/JP) 〒150 東京都渋谷区恵比寿西2丁目20番3号 Tokyo, (JP) グループ ダノン(GROUPE DANONE)(FR/FR) 75008 パリ, リュウ・ドウ・テヘラン, 7 Paris, (FR) (72) 発明者: および (75) 発明者/出願人 (米国についてのみ) 増山明弘(MASUYAMA, Akihiro)(JP/JP) 〒227 神奈川県横浜市青葉区奈良町2913-6-303 Kanagawa, (JP) 安井正明(YASUI, Masaaki)(JP/JP) 〒226 神奈川県横浜市緑区いぶき野53-8 ファミリール105 Kanagawa, (JP)</p>		<p>(74) 代理人 弁理士 酒井 一(SAKAI, Hajime) 〒102 東京都千代田区麹町5丁目7番地 秀和紀尾井町TBRビル Tokyo, (JP) (81) 指定国 AU, BG, BR, CA, CN, CZ, HU, KR, MX, PL, RO, SG, SI, SK, TR, US, 欧州特許 (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). 添付公開書類 国際調査報告書</p>
<p>(54) Title: ANTI-STRESS DRUGS AND FUNCTIONAL FOODS HAVING ANTI-STRESS EFFECTS (54) 発明の名称 抗ストレス剤及び抗ストレス作用を有する機能性食品 (57) Abstract Anti-stress drugs which can be continuously administered without any problem in safety and relieve or prevent mental and physical symptoms caused by stresses and functional foods having anti-stress effects. The drugs contain as the active ingredient acid milks prepared by fermenting animal milks by using lactic acid bacteria belonging to the genus <i>Lactobacillus</i>, which the functional foods contain these drugs and have anti-stress effects.</p>		

ABSTRACT OF THE DISCLOSURE

An anti-stress agent and functional food containing the anti-stress agent and having an anti-stress effect, which contain as an effective ingredient fermented sour
5 milk prepared by, for example, fermenting animal milk starting material with lactic acid bacteria of the genus Lactobacillus, which can be taken repeatedly and daily without any problems with safety, and which can mitigate and prevent mental and physical symptoms caused by
10 stress.

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SPECIFICATION

Anti-stress Drugs and Functional Foods having Anti-stress Effects

5 Field of Art

This invention relates to an anti-stress agent having effects of preventing and mitigating mental and physical symptoms caused by stress, and functional food containing the anti-stress agent which is prepared by
10 adding the anti-stress agent to yogurt, milk-containing acidified beverages, cheese, various processed foods, healthy foods, powdered foods, or granulated foods to give an anti-stress effect thereto.

Background Art

15 In the modern society, people undergo various kinds of stress caused by highly advanced and complicated scientific technology, or drastically changing social circumstances. Particularly, in the internationalized society, complex human relationships are formed, causing
20 mental stress. It has been reported that a variety of symptoms are caused by mental stress.

It is recognized that mental stress has a great influence on circulatory system. However, the scientific concept and definition of stress have not yet been well established, so that means of evaluation of



stress still have many problems, combined with methodological difficulties. However, in the recent years, studies of stress have been made from the medical point of view.

5 For example, it is reported that when one undergoes stress, angiotensins II and/or vasopressin increase, and intracorporeal sodium due to sodium reabsorbancy becomes excess, which causes rise in blood pressure (Osamu Mobara et al.: Taisha, 28, 2, 323, 1991). However, suffering
10 stress not only causes rise in blood pressure, but also influences various factors, such that it is believed to cause stomach ulcer, ischemic cardiac diseases, cerebrovascular diseases, hypertension, hyperlipemia, or the like. Therefore, though the investigation of the
15 relationship between stress and hypertension is of importance, it is not believed that mere lowering of blood pressure will bring about the anti-stress effect.

As an agent for preventing and mitigating mental and physical symptoms caused by stress, chemically
20 synthesized medicaments such as a tranquilizer, an antianxiety agent, and sleeping pills are presently used. However, these medicaments have habituation and side effect problems, so that it is not preferable to use them daily for the purpose of preventing mental and physical
25 symptoms caused by stress. Accordingly, foods having



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the anti-stress effect are desired and are under development, which can be taken repeatedly and daily without any problems with safety, and which can mitigate and prevent mental and physical symptoms caused by stress. For example, an anti-stress agent containing as an effective ingredient L-theanine contained in tea leaves, is proposed in Japanese Laid-open Patent Application No. 6-100442, which can be mixed in tasty beverages (nutrient supplementary drinks) for daily uptake. Further, a stress relieving effect of fragrance is also reported (FRAGRANCE JOURNAL: 1991-11, 44). However, there has not been reported that lactic acid bacteria-fermented milk has the effect of mitigating and preventing mental and physical symptoms caused by stress.

Disclosure of the Invention

It is therefore an object of the present invention to provide a functional food or drug which can fulfill the social demand as mentioned above, which can be taken repeatedly and daily without substantial problems with safety, and which may mitigate and prevent mental and physical symptoms caused by stress; and its use.

Other objects of the present invention will become apparent from the following detailed description.

The present inventors have made intensive studies to find a substance which can fulfill the social demand as mentioned above, which can be used in food, and which can be taken repeatedly without any problems with safety. As a result, they have noticed that fermented sour milk has a superior anti-stress effect, and completed the present invention.

According to the present invention, there is provided a functional food or drug when used in a method of treatment of human or animal stress, comprising as effective agent at least one fermented sour milk.

According to the present invention, there is further provided a functional food or drug for use as an anti-stress agent comprising as effective agent at least one fermented sour milk prepared by fermenting an animal or vegetable milk



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starting material with lactic acid bacteria of the strain *Lactobacillus helveticus* deposited with the accession number ATCC55796.

According to the present invention, there is also
5 provided a method of treatment of stress of a human or animal in need of such treatment comprising administering to such a human or animal an effective amount of a functional food or drug as defined above.

According to another embodiment of the invention there is
10 provided use of at least one fermented sour milk in the manufacture of a functional food or drug for the treatment of stress in a human or animal.

Brief Description of the Drawings

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15 diastolic blood pressure during the testing period of

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the mental arithmetic test in Experiment 1 in Example 1. The ordinate of the graph expresses the diastolic blood pressure in mmHg, while the abscissa expresses the time in minute.

5 Fig. 2 is a graph showing the change in Profile of Mood State (POMS) after the termination of the mental arithmetic test in Experiment 1 in Example 1. The ordinate of the graph expresses the change in POMS score in percent (%).

10 Fig. 3 is a graph showing the fluctuation in the diastolic blood pressure during the testing period of the mental arithmetic test when the panels were given fermented sour milk beverage for one week in Example 1. The ordinate of the graph expresses the diastolic blood
15 pressure in mmHg, while the abscissa expresses the time in minute.

Fig. 4 is a graph showing the fluctuation in the heart rate during the testing period of the mental arithmetic test when the panels were give fermented sour milk
20 beverage for one week in Example 1. The ordinate of the graph expresses the heart rate per minute in beat/min., while the abscissa expresses the time in minute.

Preferred Embodiments of the Invention

The anti-stress agent of the present invention contains at least one fermented sour milk as effective



agent having an anti-stress effect. The anti-stress effect of this anti-stress agent can be confirmed, employing the rise in blood pressure, the rise in heart rate, and the change in Profile of Mood State (POMS),
5 or the like as the indices, by determining the suppressing effects of the agent upon such rises and changes taken before and after the intake of the agent.

As the fermented sour milk contained as effective agent in the anti-stress agent of the present invention,
10 lactic acid bacteria-fermented milk is used, of which great safety in its repeated daily use has conventionally been confirmed. The fermented sour milk may be prepared, for example, by first preparing a milk-containing stock solution.

15 The milk contained in the milk-containing stock solution may be of animal or vegetable origin. For example, animal milk such as cow's milk, goat's milk, sheep's milk, or horse's milk; or vegetable milk from soybeans or the like, may be used. The milk starting
20 material may be whole fat or skim milk, whey, powdered milk, and/or reconstituted milk.

The milk-containing stock solution is not limited to a liquid wherein milk is dissolved or suspended and dispersed. The stock solution may be a solution-
containing material such as paste prepared by mixing milk



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powders or a milk-containing material with water or a solution of salts. Additionally, a medium for lactic acid bacteria, yeast extracts, vitamins, minerals, sugars, lipids, flavors, or coloring agents may optionally be contained in the milk-
5 containing stock solution.

Subsequently, the milk-containing stock solution is fermented with lactic acid bacteria, or symbiotically fermented with lactic acid bacteria and yeast for the purpose of improving flavor of the produced functional food, thereby
10 obtaining the fermented sour milk.

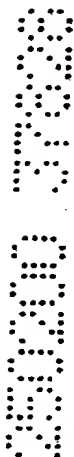
The lactic acid bacteria are preferably lactic acid bacteria of the genus *Lactobacillus*. For example, *Lactobacillus helveticus*, *Lactobacillus delbruekii* subsp. *bulgaricus*, *Lactobacillus acidophilus*, *Lactobacillus*
15 *fermentum*, or *Lactobacillus casei* subsp. *casei* may be used. Specifically a strain such as *Lactobacillus helveticus* ATCC55796 (deposited on 2 July 1996 with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852, USA as the re-deposit of deposit No. ATCC15009), *Lactobacillus*
20 *delbruekii* subsp. *bulgaricus* ATCC11842, *Lactobacillus acidophilus* ATCC4356, *Lactobacillus fermentum* ATCC14931, or *Lactobacillus casei* subsp. *casei* ATCC393 may be used. Among these, *Lactobacillus helveticus* is particularly preferred.

The yeast which can be used for symbiotic fermentation
25 with the lactic acid bacteria may be yeast of the genus *Saccharomyces*, genus *Candida* such as *Candida utilis* ATCC8205 (deposited with the American Type Culture Collection and available since at least 1990), or the genus *Kluyveromyces*. For example, a strain such as *Saccharomyces cerevisiae*,
30 *Candida utilis*, or *Kluyveromyces marxianus* var. *lactis* may be used.

Culture conditions for fermentation include sterilizing the milk-containing stock solution under heating, cooling the sterilized stock solution to the predetermined culturing



The anti-stress agent of the present invention may be in the form of the above cultured solution per se containing the fermented sour milk as an effective ingredient, or in the form of the cultured solution from



which components other than fermented sour milk and lactic acid bacteria have suitably been separated. In either case, the lactic acid bacteria or the lactic acid bacteria together with the yeast therein are kept alive, i.e., in a live form. Alternatively, the cultured solution or the separated cultured solution may be sterilized under heating up to 80 °C to prepare an anti-stress agent in a sterilized form. Further, the above sterilized or non-sterilized cultured solution may be purified to obtain fermented sour milk; may be powdered by lyophilizing, spray drying, or drying in a drum dryer; or may further be formed into tablets using vehicles or carriers.

The anti-stress agent of the present invention may be administered orally to human or animals at any time such as before suffering stress, under stress, and after suffering stress, and may be administered even daily. The upper limit of the effective dose of the anti-stress agent is not particularly limited, and may suitably be selected. But the effective dose for sufficiently mitigating and preventing stress is, when the anti-stress agent is administered to human, preferably not less than 0.1 g per kilogram of body weight per day in terms of dried product of the fermented sour milk.

The functional food having the anti-stress effect



of the present invention contains the above-mentioned anti-stress agent. Therefore, the anti-stress agent contained therein may be in the form of the cultured solution per se as mentioned above or after the suitable
5 processing, with the lactic acid bacteria or the lactic acid bacteria together with the yeast being alive; in the sterilized form prepared by sterilizing under heating up to 80 °C; or in the powdered form prepared by lyophilizing, spray drying, or drying in a drum dryer.

10 The functional food of the present invention is not particularly limited as long as it contains the anti-stress agent as mentioned above. The anti-stress agent may be added after the food production, during the food production, or at any stage of the food production.

15 Further, the functional food of the present invention may suitably contain sugars, proteins, lipids, vitamins, minerals, flavors, or coloring agents in addition to the anti-stress agent, depending on the type of the food.

The amount of the anti-stress agent contained in the
20 functional food of the present invention is not particularly limited, but is usually in the preferred range of 10 to 100 w/w% in terms of fermented sour milk.

The functional food of the present invention may be in the form of yogurt, milk-containing acidified beverages, cheese, processed foods containing fermented



sour milk, healthy foods, or powdered or granulated foods.

The effective amount of the functional food of the present invention for mitigating and preventing stress is, in the case of human, preferably not less than 0.1 g per kilogram of body weight per day in terms of dried product of the fermented sour milk.

The anti-stress agent and the functional food of the present invention can be used for manufacture of a drug for the treatment of stress in the form of solid or liquid, specifically, in the form of tablets, granules, or nutrient supplementary drinks.

The anti-stress agent and the functional food having the anti-stress effect of the present invention contain as effective agent fermented sour milk prepared by fermentation with lactic acid bacteria. Accordingly, they are highly safe, can be ingested repeatedly and daily, and have effects of mitigating and preventing mental and physical symptoms caused by stress.

20 Examples

The present invention will now be explained in more detail with reference to Examples, but the present invention is not limited thereto.

Example 1

2 kg of skim milk (solid content of 9 weight %)



sterilized by heating up to 90 °C were inoculated with
40 g of a starter of previously cultured *Lactobacillus*
helveticus ATCC55796, and cultured at 37°C for 24 hours
to prepare the secondary starter. Next, 4.5 kg of skim
5 milk powders were dissolved in 45.5 kg of water, and the
resulting solution was sterilized by heating up to 90 °C
and cooled down to the room temperature. Then the
solution was inoculated with the secondary starter, and
cultured at 37 °C for 24 hours to obtain about 52 kg of
10 fermented sour milk. After sterilizing the fermented
sour milk at 80 °C for 10 minutes by heating, Aspartame
(trade name, manufactured by Ajinomoto K.K.) was admixed
to the fermented sour milk in an amount of 0.04 weight %
of the total weight for facilitating drinking, thereby
15 obtaining an anti-stress agent. As to the obtained
anti-stress agent, the following experiments were
conducted.

Experiment 1

The above mentioned anti-stress agent and a control
20 consisting of a mixture of skim milk and lactic acid of
the same concentration as the fermented sour milk, were
given as a drink to fifteen (15) panels of healthy
individuals (7 males, 8 females, age of 24 to 32), and

mental arithmetic test as described below was conducted
25 on the panels.



The panels were given one of the anti-stress agent and the control in an amount of 100 g each at 6:00 p.m. and 12:00 p.m. on the day before the measurement, 7:00 a.m. on the day of the measurement, and 30 minutes before the commencement of the test (10:00 a.m.), i.e. a total of four (4) times in a total amount of 400 g. Then on a different set of days, the panels were given the other of the anti-stress agent and the control according to the same timetable. The tests were conducted as a blind test so that the order of doses would not affect the test results. In order to adapt the panels to the testing environment, the panels entered the testing room ten minutes before the commencement of the test, and calmed down to resting conditions before the test was started. When the test was started, the panels were kept under the resting conditions for fifteen (15) minutes, and then placed under the calculation work for thirty (30) minutes from the fifteenth (15th) minute to the forty-fifth (45th) minute from the commencement of the test. During the testing time (from 0 minute to the 45th minute), the blood pressure of the panels was measured continuously. Further, before the commencement of the test and after the termination of the calculation work, the panels were evaluated according to Profile of Mood State (POMS) method, which can evaluate the psychological conditions



with the passage of time, in order to know their psychological conditions. The results of the tests were shown as follows: as to the blood pressure, by the change from the blood pressure in the resting condition; and
5 as to the POMS, by the change from the points before the commencement of the test. The statistical differences were determined by paired t-test.

The fluctuation in the diastolic blood pressure during the testing period (from 0 minute to the 45th
10 minute) is shown in Fig. 1. The diastolic blood pressure was elevated with the load of calculation work. In the case wherein the panels were given the anti-stress agent of the present invention, the rise in the diastolic blood pressure during loading of the calculation work stress
15 was suppressed. Particularly, at the twentieth (20th) minute after the commencement of the test, the rise in the diastolic blood pressure was significantly suppressed as compared to that in the case of the controls with the significance level of 5 % (*).

20 The results of POMS test are shown in Fig. 2. It was recognized that, by comparing the results of the POMS test before the commencement of the test and after the calculation work, tension and depression tended to be relaxed, fatigue tended to be mitigated, and emotional derangement tended to be stabilized, in the case wherein



the panels were given the anti-stress agent of the present invention. In particular, tension was significantly relaxed as compared to the controls with the significance level of 5 % (*).

5 Experiment 2

Four (4) panels of healthy individuals (3 males, 1 female, age of 25 to 35) were given the anti-stress agent as a drink as mentioned above for seven (7) days in a row, and the mental arithmetic test was conducted before
10 and after the uptake of the anti-stress agent.

On the first (1st) day, the mental arithmetic test was conducted on the panels from 10:30 a.m. in the same way as in Experiment 1. Subsequently, the panels were given 100 g of the anti-stress agent in the afternoon
15 of the same day. From the second (2nd) day through the sixth (6th) day in a row, they were given 100 g of the anti-stress agent in the morning and in the afternoon. On the seventh (7th) day, the panels were given 100 g of the anti-stress agent at 7:00 a.m., and the same mental
20 arithmetic test as in Experiment 1 was conducted on the panels from 10:30 a.m. The results of the test were shown by the change in the blood pressure and the heart rate from their resting conditions. The statistical differences were determined by paired t-test.

The fluctuation in the diastolic blood pressure



during the testing period (from 0 minutes to the 45th minutes) is shown in Fig. 3. The diastolic blood pressure was elevated with the load of calculation work. In the test after the panels were given the anti-stress agent of the present invention for one week in a row, the rise in the diastolic blood pressure during loading of the calculation work was suppressed as compared to that in the test conducted before the panels were given the anti-stress agent. Further, the fluctuation of the heart rate during the testing period (from 0 minute to the 45th minute) is shown in Fig. 4. The heart rate was elevated with the load of the calculation work. In the test after the panels were given the anti-stress agent of the present invention for one week in a row, the rise in the heart rate during loading of the calculation work was suppressed, as compared to that in the test conducted before the panels were given the anti-stress agent.

Example 2

The fermented sour milk prepared in Example 1 was sterilized under heating at 80 °C for 10 minutes. After that, starting materials were mixed to obtain a mixture having the composition of 75 weight % of the sterilized fermented sour milk, 13.3 weight % of 3 wt% HM pectin solution, 3.03 weight % of 30 wt% sodium citrate solution, 4.5 weight % of 1 wt% Aspartame solution, 0.25 weight %



of blended flavor, and 3.92 weight % of water. The mixture was sterilized by heating up to 85 °C, and then charged in brown bottles by 100 g each while hot, thereby producing fermented sour milk beverage. The obtained
5 fermented sour milk beverage was subjected to the tests as in Example 1 to reveal that it has similar effects on blood pressure, heart rate, and POMS.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.



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THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A functional food or drug when used in a method of treatment of human or animal stress, comprising as effective agent at least one fermented sour milk.
2. The functional food or drug according to claim 1 wherein said fermented sour milk includes fermented sour milk prepared by fermenting an animal or vegetable milk starting material with lactic acid bacteria of the genus *Lactobacillus*.
3. The functional food or drug according to claim 2 wherein said lactic acid bacteria of the genus *Lactobacillus* are *Lactobacillus helveticus*.
4. The functional food or drug according to claim 3 wherein said *Lactobacillus helveticus* is of a strain deposited with the accession number ATCC55796.
5. The functional food or drug according to any one of claims 1 to 4 wherein said fermented sour milk includes a fermented sour milk prepared by fermenting an animal or vegetable milk starting material with both lactic acid bacteria of the genus *Lactobacillus* and a yeast.
6. The functional food or drug according to any one of claims 1 to 5 wherein said fermented sour milk is in a live form.
7. The functional food or drug according to any one of claims 1 to 5 wherein said fermented sour milk is in a sterilised form.



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8. The functional food according to any one of claims 1 to 7 wherein content of said effective agent is 10 to 100 w/w% in terms of the fermented sour milk.
- 5 9. The functional food according to any one of claims 1 to 8 wherein said functional food is in the form selected from the group consisting of yoghurt, milk-containing acidified beverages, cheese, processed foods containing fermented sour milk, healthy foods, powdered foods and granulated foods.
- 10 10. The functional food according to any one of claims 1 to 9 further comprising a component selected from the group consisting of sugars, proteins, lipids, vitamins, minerals, flavors, coloring agents and mixtures thereof.
- 15 11. A method of treatment of stress of a human or animal in need of such treatment comprising administering to such a human or animal an effective amount of a functional food or drug as claimed in any one of claims 1 to 10.
- 20 12. The method according to claim 11 wherein the functional food or drug is orally administered at a dosage of not less than 0.1g per kilogram of bodyweight per day in terms of dried product of the fermented sour milk.
- 25 13. Use of at least one fermented sour milk in the manufacture of a functional food or drug for the treatment of stress in a human or animal.
- 30 14. The use according to claim 13 wherein the fermented sour milk includes fermented sour milk prepared by fermenting an animal or vegetable milk starting material with lactic acid bacteria of the genus *Lactobacillus*.



- 20 -

15. The use according to claim 14 wherein the lactic acid bacteria of the genus *Lactobacillus* are *Lactobacillus helveticus*.

5 16. The use according to claim 15 wherein the *Lactobacillus helveticus* is of a strain deposited with the accession number ATCC55796.

10 17. The use according to any one of claims 14 to 16 wherein the fermented sour milk includes fermented sour milk prepared by fermenting an animal or vegetable milk starting material with both lactic acid bacteria of the genus *Lactobacillus* and a yeast.

15 18. The use according to any one of claims 14 to 17 wherein the fermented sour milk is in a live form.

19. The use according to any one of claims 14 to 17 wherein the fermented sour milk is in a sterilised form.

20 20. The use according to any one of claims 14 to 19 wherein the drug or functional food is orally administered at a dosage of not less than 0.1g per kilogram of bodyweight per day in terms of dried product of the fermented sour milk.

25 21. The functional food or drug according to claim 5 wherein said yeast is of a strain deposited with the accession number ATCC8205.

30 22. The use according to claim 17 wherein said yeast is of a strain deposited with the accession number ATCC8205.



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23. The functional food or drug according to claim 1, the method of treatment of stress of a human or animal according to claim 11 and the use according to claim 13 substantially as hereinbefore described with reference to the examples.

5

DATED this 25th day of February, 2000

Calpis Co., Ltd. AND Groupe Danone

10 By DAVIES COLLISON CAVE
Patent Attorneys for the Applicant

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Fig. 1

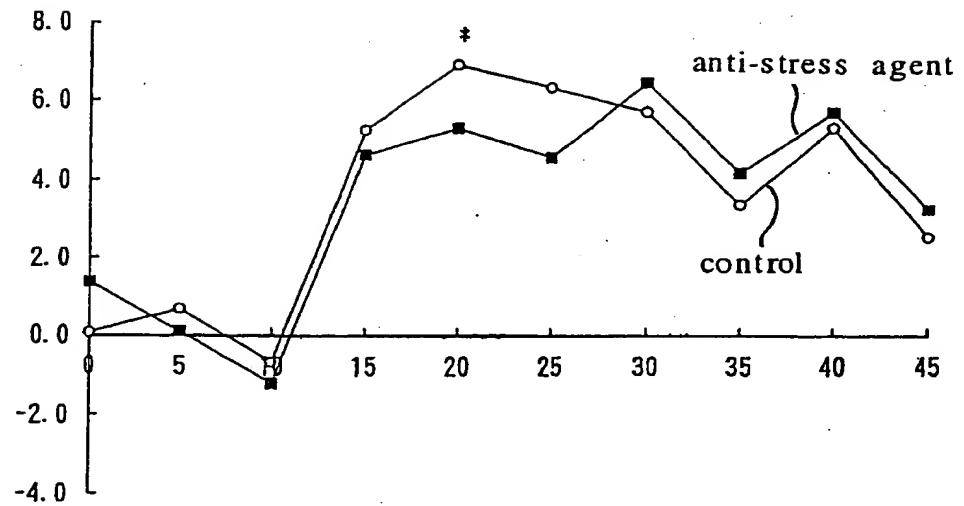


Fig. 2

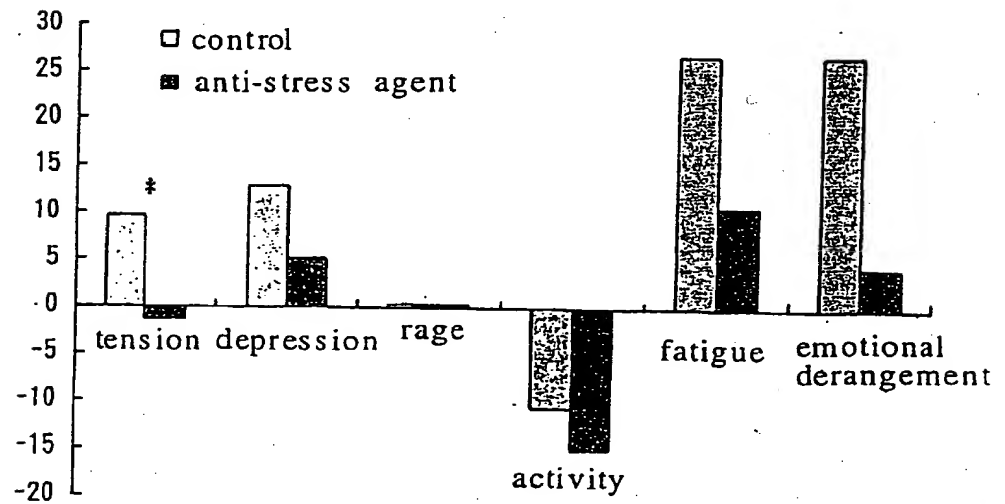


Fig. 3

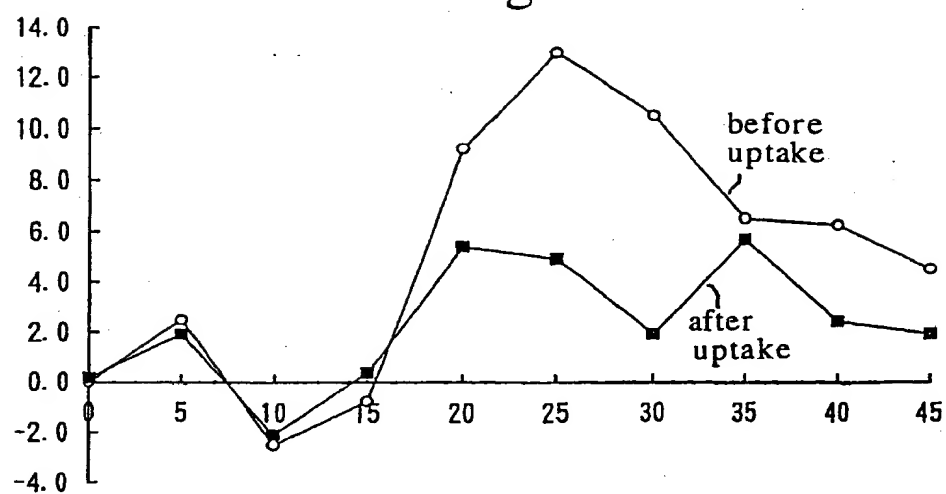


Fig. 4

